Title: Quantitative genetics of preference and performance on chickpeas in the noctuid moth, *Helicoverpa armigera*

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Abstract

If a novel, resistant host plant genotype arises in the environment, insect populations utilising that host must be able to overcome that resistance in order that they can maintain their ability to feed on that host. The ability to evolve resistance to host-plant defences depends upon additive genetic variation in larval performance and adult host-choice preference. To investigate the potential of a generalist herbivore to respond to a novel resistant host we estimated the heritability of larval performance in the noctuid moth, *Helicoverpa armigera*, on a resistant and a susceptible variety of the chickpea, *Cicer arietinum*, at two different life-stages. Heritability estimates were higher for neonates than for third-instar larvae suggesting that their ability to establish on plants could be key to the evolution of resistance in this species, however further information regarding the nature of selection in the field would be required to confirm this prediction. There was no genetic correlation between larval performance and oviposition preference, indicating that female moths do not choose the most suitable plant for their offspring. We also found significant genotype by environment interactions for neonates (but not third-instar larvae), suggesting that the larval response to different plant genotypes is stage-specific in this species.

Introduction

The appearance of a novel, resistant plant genotype in the environment presents a challenge to its herbivores; insect populations utilising that host must be able to evolve resistance in order that they can maintain their ability to feed on that host. To predict how an insect species will respond to the emergence of a new, resistant host genotype we need to know three things. First, is there additive genetic variation in the insect population for the ability to utilise the resistant host genotype? A number of studies have estimated levels of additive genetic variation in host use in herbivorous insects, the majority of which consider variation in the ability of a generalist herbivore to feed on different host species (Via, 1984a,b; Ward, *et al.*, 1993; Carriere and Roitberg, 1994; Fox and Caldwell, 1994; Hawthorne and Via, 1994; Sheck and Gould, 1996; Thompson, 1996; Tucic, *et al.*, 1997; Ueno, *et al.*, 1997; Bossart, 1998; Hawthorne, 1998; Lazarevic, *et al.*, 1998; Gu, *et al.*, 2001; Poore and Steinberg, 2001).

Fewer studies examine genetic variation in the ability of a generalist to feed on novel resistant varieties of an existing host, though this is important in terms of the ability of a generalist to maintain its host range. One such study examined variation in the ability of the polyphagous leafminer, *Liriomyza trifolii* to feed on a resistant genotype of an existing host, the chrysanthemum (Hawthorne, 1998). Genetic variation for performance on resistant chrysanthemums was identified and 10 generations of selection for

resistance in the leafminer resulted in survival in the selected lines matching that found on susceptible chrysanthemum genotypes. Therefore, in this case, the leafminer showed the potential to respond to a novel, resistant genotype of an existing host, thus maintaining its host range.

The second thing we need to know is, if there is additive genetic variation present, at which life-stage does this variation manifest itself? For many insect species, the age at which genetic variation in host use becomes apparent could be important. For example, in many lepidopteran species neonates tend to feed on the plant upon which they hatch and so are dependent on their mothers' choice of host, whilst older larvae are able to move to neighbouring plants, potentially encountering novel plant genotypes (Zalucki, *et al.*, 2002). This could result in differing selection pressures at different life stages. Moreover, levels of additive genetic variation can also vary with age or life-stage as genes may be differentially expressed, both qualitatively and quantitatively during development (Zhu-Salzman, *et al.*, 2003). If the levels of additive genetic variation in performance differ between young and old larvae then the population level response to selection could depend more strongly on responses in one life stage over another.

Consequently, the third thing we need to know is whether there is a genetic correlation between offspring performance and adult oviposition preference. For example, if there is genetic variation in adult oviposition

preference for the resistant host genotype and this is positively genetically correlated with larval performance on the plant, then we would expect a strong response to selection in both of these of traits. However, if the adults do not recognise the new, resistant genotype as a host and do not oviposit on it, then the ability of the neonate larvae to develop on the plant may be irrelevant. A number of studies have examined the genetic correlation between preference and performance (Via, 1986; Fox, 1993; Ward, et al., 1993; Nylin and Janz, 1996; Tucic, et al., 1997; Gu, et al., 2001) but to our knowledge no previous studies have considered age-related effects on additive genetic variation in host use.

Helicoverpa armigera (Hübner) is a highly polyphagous noctuid moth. It is found on a number of agriculturally important species including the chickpea, *Cicer arietinum* (L.). Chickpea is a self-fertilising species and as such occurs as highly inbred lines. Screening of chickpea germplasm has identified a number of varieties potentially resistant to *H. armigera* (Lateef, 1985). Using the *H. armigera*-chickpea, insect-host relationship as a model system, we address the question of how a polyphagous species might respond to a novel, resistant host genotype by investigating variation in larval performance on, and adult preference for a susceptible and a resistant plant genotype. This variation was then partitioned into additive genetic and residual variance and the genetic correlation across life-stages and environments examined.

Methods

Plants

Two varieties of the chickpea *Cicer arietinum* were used in this study: Tyson and ICC506. ICC506 is a variety that has been shown to have high levels of resistance to *H. armigera* in both field and laboratory studies (Lateef, 1985). Early in December 2002, 40 seeds of each variety were sown in commercially available UC Riverside potting soil mix for use in the neonate feeding assays. One week, and one month later a further 40 seeds of each variety were sown for use in the third instar feeding assays and adult oviposition trials respectively. Thus, a large number of 6 week old, pre-flowering plants were available for each assay. All seeds were scarified prior to sowing and inoculated with rhizobium.

Helicoverpa armigera culture

The laboratory culture was founded from larvae collected from northern Western Australia and from several locations on the east coast of Australia. A previous study found that gene flow was high even between distant populations in Australia and that the effective population size was large (Daly and Gregg, 1985). It was on this basis that we decided to collect larvae from sites on the East and West coasts of Australia and outcross them, thus establishing a laboratory colony that encompassed the variation

present in the Australian population. To reduce the risk of inbreeding, eggs were collected from more than 200 adults each generation. The colony had been kept in the laboratory for 3 generations at the beginning of the experiment and was reared at 26°C with natural light, a necessary condition for breeding in this species.

Sib analysis

A full-sib/half-sib design was used to determine heritabilities of feeding performance and adult host preference (Lynch and Walsh, 1998). Fifteen virgin males were each mated to two virgin females, resulting in 30 families in total. The mated females were then placed in individual containers with access to honey water, filter paper and nappy liner on which to lay eggs. Eggs from each female were collected and allowed to hatch in plastic tubs with access to artificial diet. Immediately upon hatching, 30 neonates from each family were assigned to the neonate assay. Approximately five days after hatching, larvae were transferred to individual 25 ml plastic cups. Upon reaching the third instar 20 larvae per family were assigned to the third instar assay. The remaining larvae were allowed to pupate in the cups and upon emergence moths were assigned to the adult choice assay.

Larval performance assays

Neonates. From each family, 15 neonates were randomly assigned to the Tyson and 15 to the ICC506 treatment groups. Larvae were then placed, in groups of 5, into 200 ml plastic pots containing 10 ml of 10 g/l water agar into which the stems of 5 chickpea leaves were pushed. *H. armigera* moths lay eggs singly but will lay several eggs on a single leaf (S. Cotter pers. obs.). As such, young larvae are likely to encounter each other during feeding. Rearing neonates in groups more closely mimics larval distribution in the field than would rearing in individual containers. Chickpea leaves were collected from Tyson and ICC506 plants immediately prior to testing and randomly distributed amongst the containers. Larvae were left to feed for 5 days, after which time each surviving larva was weighed. Containers were checked daily to ensure that sufficient leaf material remained. It was not necessary to replace leaf material during the experiment.

3rd instars. From each family, 10 third instar larvae were randomly assigned to the Tyson, and 10 to the ICC506 treatment groups. Larvae were starved for two hours, weighed and then placed, individually, into 25 ml plastic pots containing 5 ml of 10 g/l water agar into which the stems of a single chickpea leaf was pushed. Older *H. armigera* larvae are more solitary and can be cannibalistic and so rearing in individual cups at this stage is necessary (S. Cotter pers. obs.). Again, chickpea leaves were

collected immediately prior to testing and randomly distributed amongst the containers. After 24 hours of feeding, larvae were weighed a second time to give an estimate of weight gain.

Adult choice assay

Five female moths from each family were mated and then placed in individual 600 ml containers with two branches of Tyson and two of ICC506 in agar. The branches were matched for size and arranged alternately in a circle around a central feeder. After 24 hours, each container was rotated to avoid any positional effects on female choice. Females were left to lay eggs for two days after which time the branches were removed and the eggs laid on each counted. Preliminary tests found that the repeatability of female choice using this experimental technique was high ($r = 0.78 \pm 0.13$, $MS_{among\ females} = 0.193$, $MS_{within\ females} = 0.024$, $n = 2$ preference measurements per female).

Variance Components Analysis

Heritability estimates of each trait and genetic correlations between traits were estimated using a multivariate restricted estimate maximum likelihood (*REML*) procedure (*VCE version 4*, Groeneveld and Kovac, 1990; see http://w3.tzv.fal.de/genetik/public_html/). This involved fitting an individual "animal model" where the phenotype of each individual was

separated into additive genetic components of variance plus other random and fixed effects, such that: $\mathbf{y} = X\mathbf{b} + Z\mathbf{a} + \mathbf{e}$. Where **y** was a vector of phenotypic values, **b** and **a** were vectors of fixed and random effects, **e** was a vector of residual values, and X and Z were the corresponding design matrices relating records to the appropriate fixed or random effects (Lynch and Walsh, 1998). The phenotypic variance of each trait, V_P is thus described as $V_P = V_A + V_M + V_R$, where V_A is the additive genetic variance, V_M is the variance attributable to maternal effects and V_R is the residual variance which includes non-additive sources of genetic variance such as dominance variance or epistatic effects, environmental effects and error variance. All of the estimates for variance due to maternal effects were non-significant and so were removed from the models. The effect of "cage" on the neonate estimates of performance was also non-significant and so was removed from the models.

The heritability of each trait was calculated as the ratio of additive genetic variance to phenotypic variance: $h^2 = V_A / V_P$. Genetic correlations between each pair of traits, $r_{A,}$ were estimated from the genetic covariance estimate between the two traits Cov [x,y], and the estimate of additive genetic variance for each trait V_{Ax} and V_{Ay} where $r_A = \text{Cov}[x,y] / [(V_{Ax})(V_{Ay})]^{0.5}$. The *VCE* program returns standard errors for all estimates, the significance of which could then be determined with t-tests. As the REML procedure assumes that the data are normally distributed, larval

weight gain data were log-transformed and percentage egg-lay data were angular-transformed prior to analysis to conform to this assumption. The analysis was then repeated with the untransformed data in order to calculate estimates for the coefficients of additive genetic and residual variance (CV_A and CV_R respectively), where $CV_A = 100\ (V_A)^{0.5} / X$ and $CV_R = 100\ (V_R)^{0.5} / X$ and X is the population mean.

Results

Larval performance and adult oviposition on each chickpea variety

The effects of plant genotype on larval performance and adult oviposition preference were analysed with linear mixed models using restricted maximum likelihood (REML) in Genstat. We included *sire* as a random effect, and *plant* and the interaction between *sire* and *plant* as fixed effects. As expected, neonate performance, measured as weight gain over 5 days feeding on chickpea, was significantly higher on the susceptible chickpea Tyson, than on the resistant variety ICC506 (Wald statistic $\chi^2 = 106.07$, df = 1, $P < 0.001$, Table 1), though there was no effect of chickpea variety on neonate survival (logistic regression, $\chi^2 = 0.36$, df = 1, $P = 0.54$).

Third instar performance, measured as weight gain over 24 hours, was also higher on Tyson though the effect was much smaller (Wald statistic $\chi^2 = 4.69$, df = 1, $P < 0.05$; Table 1); there was no mortality in third instars over the course of the feeding test. Despite the fact that the suitability of

Tyson for larval development seemed to be higher than that of ICC506, there was no significant difference between the numbers of eggs laid by adult moths on each variety (Wald statistic $\chi^2 = 0.06$, df = 1, P = 0.81, Table 1).

Heritability of larval performance

All of the heritability estimates for larval performance were highly significant (Table 1). In contrast, the heritability of innate adult host preference was not significant, though the CV_A values calculated using the untransformed data suggest that there is additive genetic variation present in this trait.

The estimates for the heritability of neonate performance on each host plant were higher than the respective third instar estimates though this was marginally non-significant for heritability of performance on Tyson (Tyson, $h^2_{neonate} = 0.441 \pm 0.050$, $h^2_{3rd\ instar} = 0.295 \pm 0.056$, $t_{28} = 1.95$, P = 0.061; ICC506, $h^2_{neonate} = 0.578 \pm 0.069$, $h^2_{3rd\ instar} = 0.344 \pm 0.060$, $t_{28} = 2.56$, P < 0.05). There was a trend for the heritability estimates of performance on ICC506 to be higher than on Tyson but this was not significant (Table 1). An examination of the CV_A and CV_R calculated for each trait show that there are similar levels of additive genetic variation present in all the measures of performance but that the levels of residual variance are higher for third instar performance (Table 1).

Genetic correlations across life-stages

Whilst there was a significant positive genetic correlation between neonate and 3rd instar performance on ICC506 ($r_A = 0.517 \pm 0.122$, $t_{13} = 4.24$, $P < 0.001$; Table 2), there was no comparable correlation across life-stages for larvae feeding on Tyson ($r_A = 0.014 \pm 0.142$, $t_{13} = 0.10$, $P > 0.05$; Table 2).

Trait variation and the environment

Trait variation across environments can be examined in two ways. Firstly, variable trait expression can be regarded as the trait itself and variation partitioned into that explained by the genotype and that explained by the environment. A significant genotype by environment interaction shows that genotypes perform relatively differently in each environment. The second approach considers trait expression in each environment as a different trait and examines the genetic covariance between them; a genetic correlation significantly lower than 1 indicates that the ranking of genotypes differs across environments (Lynch and Walsh, 1998).

Using the first approach, there was a significant genotype by environment interaction for neonate performance (Wald statistic $\chi^2 = 68.87$, $df = 28$, $P < 0.001$, Fig. 1a) but not for 3rd instar performance (Wald statistic $\chi^2 = 12.84$, $df = 28$, $P = 0.99$, Fig. 1b). The second approach confirms this

result. The genetic correlation across environments for the neonates was significantly lower than 1 (r_A = 0.198 + 0.130, t_{13} = -6.17, P < 0.001; Table 2), whereas the correlation across environments for the 3rd instar larvae was not significantly different from 1 (r_A = 0.945 + 0.049, t_{13} = -1.12, P = 0.28; Table 2).

Discussion

To predict how a generalist will respond to the emergence of a new, resistant host genotype in the environment, we need to know three things: is there additive genetic variation for the ability to utilise the resistant host genotype, at which life-stage does this variation manifest itself and is there a genetic correlation between offspring performance and adult oviposition preference?

In this study, we found moderate levels of additive genetic variation in the ability to utilise a susceptible chickpea and a novel, resistant chickpea genotype in the *H. armigera* population. It has been argued that CV_A rather than heritability should be used as an indicator of evolvability (Houle, 1992). Heritability is the measure of evolvability in the standard deviation (sd)-standardised version of the breeders equation (Lynch and Walsh, 1998), however, Hereford *et al* (2004) suggest that standardising by the standard deviation is inappropriate as the standardisation factor is itself a function of the additive genetic variance. Alternatively, standardising by the trait mean

results in the measure of evolvability being the CV_A. As such, if the mean-standardized strength of selection is equal for all measured traits, the CV_A would be the best predictor of the response to selection, whereas if the sd-standardized strength of selection is equal for all measured traits, the heritability would be the best predictor of the response to selection.

Interestingly, the heritability of performance was higher for neonates than third instar larvae, suggesting that the sd-standardised response to selection for performance on a plant should be stronger in neonates than in older larvae. In contrast, the CV_A values calculated from the untransformed data were very similar across all groups suggesting that the mean-standardised response to selection would be similar at both life-stages. It seems likely that the probability an individual will survive to reproduce in the field would be associated with its *absolute* growth rate rather than with its growth rate relative to other individuals. As such it is not possible to predict which form of standardised selection would be more comparable to that acting in the field. Therefore, in order to predict the response to selection in the field, further information regarding the nature of selection at each life stage would be required.

On the resistant chickpea genotype, ICC506, there was a strong positive genetic correlation in larval performance across the life stages. If the basis of this correlation is pleiotropy rather than linkage disequilibrium, then the genes controlling the trait at each life stage were largely the same.

Conversely, on the susceptible genotype, Tyson, there was no genetic correlation in performance across life stages. It is interesting to note that there was also a strong positive genetic correlation between neonate performance on ICC506 and third instar performance on Tyson. This poses interesting questions regarding the genes controlling larval performance on each host.

A possible explanation for the pattern of genetic correlations in larval performance could be due to constitutive versus induced resistance to plant defence mechanisms at the different life stages. Plants use a number of resistance mechanisms that can affect insect feeding including physical factors such as leaf toughness or trichome density, or chemical factors such as toxic allelochemicals and proteinase inhibitors. When encountering chemical defences, insects can respond in kind. For example, insects can detoxify allelochemicals via the inducible cytochrome P450 monoxygenase system (Berenbaum, 1991; Berenbaum, *et al.*, 1992; Rose, *et al.*, 1992; Hung, *et al.*, 1995; Scott, *et al.*, 1998; Harrison, *et al.*, 2001; Li, *et al.*, 2002), or produce or upregulate alternative proteases that are not susceptible to inhibition, or that can digest the proteinase inhibitors present in the diet (Broadway, 1996; Broadway, 1997; Wu, *et al.*, 1997; Patankar, *et al.*, 1999; Patankar, *et al.*, 2001; Moon, *et al.*, 2004).

The specific mechanism of resistance responsible for the differences between Tyson and ICC506 is unknown, however, the evidence to date

suggests resistance in chickpeas is primarily due to acid exudates on the leaf surface (Lateef, 1985) or isoflavonoids (Simmonds and Stevenson, 2001), it is therefore likely to involve some kind of secondary compound to which the feeding larva is forced to respond. Neonates have few fat reserves and establishment on a plant is critical to survival. In these circumstances, it would be beneficial for a detoxification system to be an induced response, switched on only when necessary. In other words, neonates placed on the resistant host, ICC506, faced with secondary compounds would respond by switching on genes responsible for the production of detoxifying enzymes or insensitive proteases, whilst neonates on Tyson, the more palatable host, would not.

Third instar larvae with greater fat reserves may have these genes switched on as a form of constitutive resistance, or alternately, the threshold level of gut function disruption at which these genes are switched on may be much lower than for neonates. This scenario could result in genes being switched on in all larvae tested, except for the neonates on Tyson. Further studies are necessary to determine the secondary compounds responsible for resistance in ICC506 and the mechanisms of detoxification used by *H. armigera* in response to these compounds in the diet.

In contrast to performance, adult oviposition preference was not heritable and there was no overall preference for the susceptible chickpea, Tyson (at least under the experimental protocol employed here). It may be that the

females were unable to discriminate between the two varieties of chickpea. Both lines appear to be identical and whilst there may be differences in secondary compounds between the lines these may not be detectable prior to ingestion. As such there may be no chemical cues available to a female to indicate that one line is less suitable for her offspring than another. A lack of genetic correlation between oviposition preference and offspring performance, whilst counterintuitive, is not unusual even at the level of species e.g. (Thompson, 1988 and references therein; Fox, 1993; Nylin and Janz, 1996; Gu, et al., 2001), and as such, it may be that the lack of genetic correlation in this case is accurate.

Previous studies have suggested that adult host-preference is strongly influenced by factors such as plant abundance and experience with a particular host (Papaj and Rausher, 1987; Cunningham, *et al.*, 1998, Cunningham, et al., 1999; Cunningham and West, 2001). Whether the lack of preference for either genotype of chickpea found here is due to the inability to discriminate between the two or due to a lack of experience with either host, it seems likely that females encountering a large patch of host plants, as would occur in an agricultural situation, would oviposit regardless of the genotype present. Under these circumstances, it seems likely that there would be strong selection acting on neonates for improved performance and that the response to such selection would be rapid. Future

studies should focus on the strength of selection at each life stage to further examine the potential for insect-adaptation to resistant hosts in the field.

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Titles and legends to figures

Figure 1. Genotype by environment interactions for each life stage

Family means +/- SE using the transformed data are plotted for each chickpea variety for neonates (a) and for third instars (b). Non-parallel lines are an indication of a genotype by environment interaction. Neonate growth rate is measured as log (weight in mg after 5 days +1). Third instar growth rate is measured as the residuals from the regression of weight after assay on weight before assay.

Table 1. Trait means and heritability estimates.

Trait means and heritabilities are given with their standard errors. N refers to the number of larvae sampled for each trait; there were 30 families in total. Heritabilities were estimated by partitioning the total variance into additive genetic variance and residual variance. Maternal effects were non-significant and so were removed from the model. Coefficients of additive genetic covariation (CV_A) for transformed data are meaningless (Houle, 1992), therefore CV_A and CV_R were calculated for the untransformed data. ns $p > 0.05$, *** $p < 0.001$.

Trait	Untransformed mean	Transformed mean	N	Heritability	CV_A	CV_R
Neonate						
Tyson	1.902 ± 0.083	0.949 ± 0.023	388	0.441 ± 0.056 ***	70.44	72.76
ICC506	1.102 ± 0.040	0.685 ± 0.016	395	0.578 ± 0.069 ***	59.84	57.99
3rd instar						
Tyson	12.86 ± 0.876	1.166 ± 0.871	300	0.295 ± 0.056 ***	68.61	96.58
ICC506	10.48 ± 0.747	-1.166 ± 0.745	300	0.344 ± 0.060 ***	72.26	100.14
Adult						
Host preference	0.542 ± 0.020	0.841 ± 0.026	116	0.053 ± 0.037 ns	20.47	34.60

Neonate performance, untransformed data: weight in mg after 5 days, transformed data: log (weight in mg after 5 days +1). **Third instar performance**, untransformed data: weight gain in mg over 24 hrs, transformed data: residuals from the regression of weight after assay on weight before assay. **Adult host preference,** untransformed data: eggs on Tyson/total eggs on leaves, transformed data: arcsin√(eggs on Tyson/total eggs on leaves).

Table 2. Genetic correlations between traits.

Values show genetic correlations as estimated by *VCE*. There were 30 families in total. Significance levels were determined with t-tests. ns $p <$ 0.05, ** $p < 0.01$, *** $p < 0.001$.

	Neonate -ICC506	**3rd instar -Tyson**	**3rd instar -ICC506**	**Adult host preference**
Neonate - Tyson	0.198 ns	0.014 ns	0.061 ns	-0.108 ns
Neonate - ICC506		0.393 **	0.517 ***	0.325 ns
3rd instar - Tyson			0.945 ***	0.128 ns
3rd instar - ICC506				0.038 ns

Neonate performance, transformed data: log (weight in mg after 5 days +1). **Third instar performance**, transformed data: residuals from the regression of weight after assay on weight before assay. **Adult host preference,** transformed data: arcsin√(eggs on Tyson/total eggs on leaves).